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THE CHARACTERISTICS OF BACTERIA OF THE COLON TYPE OCCURRING ON GRAINS *

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INTRODUCTION

It has been recognized for many years that bacteria of the colon-aerogenes type have a wide distribution outside of the digestive tract.

In 1899 Laurent[1] expressed the opinion that Bacillus coli communis may become parasitic on the tubers of the potato, and at about the same time Klein and Houston[2] noted the occurrence of both typical and atypical colon bacteria on grains coming from different parts of the world. Papasotiren[3] found Bacillus coli on grains of various kinds, altho it was not invariably present. Düggeli[4] found bacteria on the surface of plants, fruits, and seeds in numbers too great to be accounted for by contamination from dust of the air. Bacillus coli was among the varieties occurring less frequently. In this country, Metcalf[5] reported the occurrence of Bacillus coli on the surface of fruit, flowers, and various grains. In this work the possibility of fecal contamination was admitted; but Prescott,[6] who made an extensive study of the distribution of the organism in question on grains, found it not only on grains growing in fertilized fields, but also on grains growing under conditions which made the possibility of direct contamination with fecal matter remote. The work of Winslow and Walker[7] did not confirm the results obtained by Prescott and other investigators. No typical Bacillus coli cultures were obtained from 178 samples of grain or 40 samples of grass.

The general assumption has been that the colon-aerogenes group was widely distributed throughout nature, but that its normal habitat was the digestive tract, and its occurrence elsewhere due to a more or less direct contamination with fecal matter. It is held by many bacteriologists that multiplication is confined almost exclusively to the digestive tract, and that the occurrence on grains is due to contamination by dust particles. This view is not in accord with the work of Johnston,[8] who found a bacillus, which he could not differentiate from Bacillus coli, causing a bud rot of certain palms. Burri and Andrejew[9] believed that a strain which they found growing on grass was distinct and could be

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* Received for publication May 3, 1915.
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[1] Inst. Past. Ann., 1899, 13, p. 1.
[2] Twenty-Ninth Ann. Rep. Gt. Brit. Govt. Bd. Suppl. Rep. Med. Off., 1899-1900, p. 593.
[3] Arch. f. Hyg., 1901-1902, 41, p. 204.
[4] Centralbl. f. Bakteriol., II, 1904, 12, pp. 602, 695; 13, pp. 56, 198.
[5] Science, 1905, 22, p. 439.
[6] Ibid., 1902, 15, p. 376; Biological Studies by the Pupils of William Thompson Sedgwick, Boston, 1906.
[7] Science, 1907, 26, p. 797.
[8] U. S. Dept. of Agr., Bureau of Plant Industry, 1912, Bull. 228.
[9] Centralbl. f. Bakteriol., I, O., 1910, 56, p. 217.

distinguished from the fecal type by physiologic reactions. Prescott, while he considers the grain and fecal types identical, believes that there is actual growth on the grains. The failure to differentiate the colon-like organisms found on grain from those occurring in the intestinal tract is not surprising in view of the chaotic condition existing in the classification of this group. The varieties which have been recognized have been based on characters which are unimportant, or admittedly arbitrary.

In addition to its very apparent scientific interest, the problem of the identity or plurality of the strains of the colon-aerogenes group occurring in the intestines and on grains has a sanitary importance in relation to the significance of the occurrence of this organism in water and milk. If the surface of grains, fruits, and plants in general is the habitat of a distinct variety of this group, the mere demonstration of colon organisms in milk or water loses much of its significance, and becomes more than ever only presumptive evidence of contamination with fecal matter.

In our earlier work,[10] we have shown that while the colon bacteria occurring in bovine feces belong to one sharply circumscribed variety, there occur in milk two or more distinct physiologic families. It is true that this conclusion in regard to the bacteria of bovine feces is based on the examination of sixteen samples coming from a single herd, but the results obtained from the 150 cultures studied were so consistent that it is believed they are characteristic. In this paper, we propose to show the characteristics, particularly the physiologic functions, of a collection of colon-like bacteria obtained from representative samples of grain, and by means of these characteristics to group them into types which may be compared with the type found in the bovine digestive tract.

SOURCE OF CULTURES

Of the 166 cultures included in this study, 75 were obtained from corn, 6 from barley, 30 from wheat, and 41 from oats. These were all dried grains, obtained from the grain inspection laboratory of the Bureau of Plant Industry through the kindness of Dr. Duvel and his assistants. The samples were taken in most cases from cars of grain on the track and were transmitted to our laboratory without breaking the packages. Cultures were thus obtained from thirty-three samples of dried grains coming from five states.

In addition to the cultures from the dried grains, fourteen cultures were secured from two samples of green oats, collected in the vicinity of Washington. The heads of the grain were taken without handling and the husks opened with aseptic precautions, so that the growth came from the immature, milky grain. In the case of a third sample, transferred in a similar way to dextrose broth, no growth was obtained.

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[10] Jour. Infect. Dis., 1914, 14, p. 411; 15, p. 100.

It is obvious that there was abundant opportunity for contamination of the samples of dried grains, both from dust of the air and from the hands of the various people who handled the grain. There can be no assurance that the colon-like organisms found on these samples actually grew on the grain. In the case of the two samples of green oats, it is very probable that the bacteria were actually growing in the milk of the grain. The conclusive evidence which will be presented in this paper of the occurrence on grains of a distinct type demonstrates that these bacteria are not air-borne contamination from fecal sources.

The cultures were obtained by placing a few grains of the sample in dextrose broth, incubating at 30 C. over night, and plating the broth on infusion agar. Representative colonies were fished from the plates to dextrose broth with inverted tubes, and four or five of the tubes showing gas were replated.

Altho the fermentation of dextrose rather than lactose was used as a basis of isolation, nearly all of these cultures would respond to the usual tests for the colon group. With the exception of a small sub-group, all gave the usual presumptive tests for colon, including the formation of gas in lactose bile.

METHODS OF STUDY

Gelatin Liquefaction.—The liquefaction of gelatin was determined by spreading a drop of a broth culture on the surface of infusion gelatin in a tube, marking the level of the surface of the gelatin on strips of paper on opposite sides of the tube, and incubating for twenty days at 20 C. If liquefaction occurred, the amount was measured and expressed in millimeters of liquefaction.

Indol.—The production of indol was determined in a medium consisting of 1,000 c.c. water, 0.3 gm. of tryptophan, and 5 gm. of K_2HPO_4. Cultures were grown in this medium two days at 30 C. The test for the presence of indol was made as follows: To the medium was added, drop by drop to avoid mixing it with the meduim, 1 c.c. of a 2 percent alcoholic solution of p-dimethylamidobenzaldehyd, then a few drops of concentrated hydrochloric acid. The presence of indol was indicated by a violet color.

Capsule.—In the earlier investigations no attempt was made to determine the presence of a capsule, but on account of the importance ascribed to this characteristic by some bacteriologists, it was decided to include this in the routine examination of these cultures. With the methods at first employed, it was very difficult to get consistent results. Later we adopted the Welch method. This, as outlined to us by Dr. Harris, of the University of Chicago, is as follows: Make a smear from an eighteen-hour milk culture. Dry. Fix over the flame. Wash with glacial acetic acid. After five seconds, wash several times with carbolfuchsin. Wash well with 0.9 percent solution sodium chlorid and mount in this solution for examination. While this method was more satisfactory, it was possible to obtain consistent results only by careful manipulation in the preparation of the slides.

In certain groups, the capsule appeared large and unmistakable. This was especially true of Type E. In Type D, on the contrary, in which the capsule is recorded positive for 30 percent of the cultures, it was small and often faint and indefinite. Probably this group is constant in the possession of a small capsule which often fails to be demonstrated by the method employed.

Pigment Formation.—The members of the colon group are not usually considered as pigment formers, altho pigmented, colon-like cultures have been reported.[11] One of the cultures isolated from bovine feces was pigmented. Such a large proportion of the grain series now under consideration showed some color that it was decided to determine the pigment with more care than is usually attempted. In most of the cultures, the amount of pigment was so small, especially in the young culture, that it would not be noted, or at least would be classed as a "dirty white." In a few cultures, however, the color was distinct, even in the young culture.

The pigment was compared with a standard by growing an agar culture fifteen days at 20 C. and spreading as much as possible of the growth on white drawing paper. Better results were obtained by comparing the standard with the moist culture than with one which had dried on the paper, because the amount of pigment was so small that when dried it did not cover the white of the paper, and it was difficult to get consistent results.

The standards used were the plates in Ridgway's Color Standards and Nomenclature, which have been found valuable for this purpose. A word of explanation of the symbols used to designate these standards may make Table 7 more intelligible. Colors are designated by letters, as "oy," which indicates a color made up of 65 percent orange and 35 percent yellow; "yoy," 47 percent orange and 53 percent yellow; or "oyy," 25 percent orange and 75 percent yellow. Variations from the pure colors are obtained by mixtures of neutral gray. For instance, 17oy is a pure color; 17′oy contains 32 percent neutral gray; 17″oy, 58 percent neutral gray. Finally, tints, designated by lower-case letters, are obtained by mixing varying proportions of white with the pure colors, and shades are obtained by mixing black. The tint nearest the pure color is designated by the letter b, that nearest pure white by f; the shade nearest the pure color is designated by the letter i.

Reference to the plates will show that the difference between the corresponding lighter tints of the various orange-yellow colors is so small that the identification of the pigment of a culture with one or the other does not indicate a marked difference. In making up Table 7, only the colors, tints, and shades were included with which cultures were identified.

Fermentation.—The value of the fermentation reactions for the division of the colon-aerogenes group into its component sub-groups is so well established that it need not be discussed in this paper. There is much uncertainty and difference of opinion, however, as to the test substances to be used in fermentation tests. In our opinion, no definite schedule of test substances can be predetermined, as the particular ones giving the most characteristic reactions will vary with the group of cultures under observation. These should include representatives of the different types of compounds which may serve as sources of carbon for zymogenic bacteria. Therefore we included simpler sugars, such as dextrose; more complex sugars, such as saccharose, lactose, and raffinose; polysaccharids, as starch and inulin; alcohols, such as mannite, glycerin, adonite, and dulcite; and one glucosid, salicin.

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[11] Centralbl. f. Bakteriol., II, 1914, 41, p. 1.

The fermentation of a substance may be determined by the evolution of gas or by the production of acid. In our work, we have confined the study of fermentation by gas production to dextrose, and have depended on the acidity determination for the fermentation of other test substances.

Under certain circumstances which have not yet been definitely determined, the acid from the fermentation of sugar may be masked by a secondary alkaline production, sufficient in some cases entirely to obscure the acid formation. In one small group of this collection, the lactose broth tubes at the end of seven days were only slightly more acid than the blank, altho all of the cultures gave gas in lactose bile. In no case was the titration of the culture less than that of the blank, altho this was usually the case with broths in which there was no fermentation. Where positive evidence of the fermentation of a sugar was obtained in another way, the negative evidence of the titration was disregarded and in the correlation tables the culture was included with the positive reactions. If, for instance, the titration of lactose broth was negative, while the lactose bile fermentation tubes showed gas, the cultures were considered to be lactose positive.

Kligler[12] considers the reaction with salicin very significant. We included this glucosid with the test substances used in some of our earlier work, but did not use it on the fecal series because we found that it was fermented by such a large percentage of the cultures that it seemed to have little value for diagnostic purposes. Altogether, 279 cultures were tested for their ability to ferment salicin. Of these 264, or 94.6 percent, fermented salicin while only fifteen failed. Notwithstanding this evidence, salicin was included in this series in order that the results might be more comparable with those obtained by Kligler. It was found however that only one or two of the entire collection failed to ferment salicin. In compiling the tables, the results obtained with salicin and dextrose were omitted for the sake of brevity.

In studying the gas production of these bacteria, we again used the mercury vacuum pumps and the technic described in our former papers.[13] The only modification of the procedure described in our last paper was the use of a Sprengel pump to complete the initial evacuation of the bulbs begun with a "Geryck" oil pump. The gas was analyzed over mercury in carefully calibrated burettes. All gas volumes were reduced to normal temperature and pressure dry.

The medium was of the same composition as that employed in our last series of determinations with the colon cultures from cow feces. It was made as follows: Ten grams dextrose, 10 gm. Witte's peptone, and 5 gm. Kahlbaum's dibasic potassium phosphate (K_2HPO_4) were added to 800 c.c. distilled water. This mixture was heated with occasional stirring for twenty minutes over steam. It was then filtered through a Schleicher and Schüll No. 588 folded filter. The filtrate was cooled to 20 C. and then made up to 1,000 c.c. The dextrose with which this medium was made up, was, in this series, a sample of exceptionally high purity given us by Dr. Hudson of the Bureau of Chemistry.

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[12] Jour. Infect. Dis., 1914, 15, p. 187.

[13] Jour. Infect. Dis., 1914, 14, p. 411; 15, p. 100.

The incubation temperature was 30 C., maintained constant ± 0.1°, except on two occasions both of which fortunately occurred at the end of an incubation period. We did not strictly adhere to the seven-day incubation period which we used in former series because, in every case we have studied, gas production by similar organisms ceased within one hundred hours after inoculation. A variation of one or two days from the seven day incubation period is therefore not likely to have affected our results.

All analyses made, except four, are included in the tables. In two of the four omitted, gross errors were definitely detected in the determination of carbon dioxid. The total gas in these cases was for *qy,* 28.8 c.c., and for *op,* 27.8 c.c. In the other two analyses, gross errors in the determination of hydrogen were definitely detected. The carbon dioxid contents of these cases were for *qq,* 69.8 percent and for *qe,* 71.4 percent. For the duplicate determinations with these organisms, see Table 3.

The separation of the analyses into three tables was made upon the basis of very distinct differences in the ratio $CO_2:H_2$. In Table 1 are grouped the eight cultures the ratios of which were low, agreeing with those of that large group previously isolated by us from bovine feces.

In Table 2 will be found the analyses of gas from seven cultures which produced carbon dioxid but no hydrogen. The ratio $CO_2:H_2$. in these cases became infinity (∞—a symbol which we shall retain in spite of its pedantry for its convenience in tabulation and description.)

All of the remaining cultures gave a ratio above 1.90, their general characteristics being tabulated in Table 3. The cultures are subdivided in this table into the four types which will be described later. Under each type are placed, first, those cultures which showed very little or no variation from the type, and next, the cultures which differed from the type in one or more significant characteristics. We shall therefore refer to the cultures and analyses which fall in Tables, 1, 2, or 3 as members of the "low ratio group," "∞ group," or the "high ratio group," respectively.

The averages of the values listed in the three tables of analyses are given in Table 4, which is self-explanatory in all points except the "nitrogen" values. These were calculated by subtracting the sum of carbon dioxid and hydrogen from the total volume and assuming that the residual gas was nitrogen.

In order to visualize the distinct differences in the gas production of these three groups, the individual analyses have been plotted in Chart 1. In this chart, the abscissa of each dot represents the cubic centimeters of CO_2 produced, and the ordinate the cubic centimeters of H_2. The diagonal passing through the origin is the $1.06\ x = y$ line along which

TABLE 1

Characteristics of Cultures with Ratio $CO_2/H_2 = 1.06$ (Type A)

Culture	Capsule	Liquefaction of Gelatin	Indol from Tryptophan	Pigment	Fermentation Reactions Expressed as c.c. Normal Acid per 100 c.c. Medium									Volume of Gas in c.c.	Percentage CO_2	Percentage H_2	Ratio $\frac{CO_2}{H_2}$
					Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite				
oq	—	0	+	19′yoya	—1.30	6.10	3.65	—0.60	0.00	4.15	1.70	4.80	4.30	11.80 9.72	51.0 51.5	48.1 48.2	1.06 1.07
or	+	0	+	19′yoyb	6.60	5.00	4.70	—0.70	0.00	4.20	1.05	0.00	—1.20	8.03 8.04	51.9 50.7	47.4 47.9	1.09 1.06
ou	—	0	—	19′yoya	—0.70	4.20	0.90	0.00	0.00	3.45	0.95	0.00	0.00	12.58 11.94	51.4 51.8	48.5 48.4	1.06 1.07
st	—	0	—	19′yoya	0.70	0.70	2.30	0.00	—1.10	2.80	0.75	0.00	—1.35	15.06 16.75	50.8 51.5	48.8 48.5	1.04 1.06
sv	—	0	—	19′yoya	0.90	0.00	3.00	0.00	0.00	3.35	0.60	—0.95	—1.10	14.67 16.02	51.5 51.0	48.2 48.6	1.06 1.05
te	—	0	+	19′yoya	—0.25	2.35	1.35	—0.30	—0.85	3.80	0.95	4.25	3.70	8.51 12.00	52.0 51.4	47.5 48.0	1.10 1.07
tf	—	0	—	19yoya	3.85	1.95	2.55	0.00	3.85	3.10	0.75	0.00	1.55	15.41 15.82	51.9 50.7	47.9 49.2	1.08 1.03
ue	—	0	+	19′yoyi	—0.85	4.70	0.35	—0.30	—0.95	4.00	0.55	3.85	3.40	10.44 11.81	50.9 50.8	48.6 48.3	1.05 1.05

TABLE 2

Characteristics of Cultures Producing CO_2 Only

Culture	Capsule	Liquefaction of Gelatin in mm.	Indol from Tryptophan	Pigment	Fermentation Reactions Expressed as c.c. Normal Acid per 100 c.c. Medium									Volume of Gas (CO_2) in c.c.	Residual Gas
					Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite		
pl	—	7	+	17oya	4.30	4.15	3.20	—0.20	—0.75	3.05	3.55	—0.55	—1.15	4.47 3.45	Trace Trace
tj	—	6	—	19′yoya	3.30	—0.15	0.00	—0.05	—0.55	3.15	0.65	—0.60	—1.30	3.15 3.31	Trace Trace
tk	—	8	—	17oya	3.40	—0.20	0.00	—0.05	—0.70	2.85	0.70	—1.05	—1.20	5.96 3.49	Trace Trace
uy	+	30	—	17′oyf	4.95	0.45	—0.65	0.05	—0.50	4.20	4.15	3.20	—0.75	8.87 8.31	Trace Trace
vd	—	37	—	19′yoyf	5.00	0.60	—0.45	0.40	—0.45	2.45	4.50	3.35	—0.35	7.44 7.78	Trace Trace
ve	—	44	—	17″oyd	4.85	0.70	0.00	—0.05	—0.35	3.55	3.65	5.35	—0.60	3.96 5.10	Trace Trace
vf	—	43	—	17″oyd	5.85	0.45	0.00	0.00	—0.75	3.80	3.75	3.60	—0.60	5.14 6.60	Trace Trace

TABLE 3

Characteristics of Cultures with CO_2/H_2 Above 1.90

Culture	Capsule	Liquefaction of Gelatin in mm.	Indol from Tryptophan	Pigment	Fermentation Reactions Expressed as c.c. Normal Acid per 100 c.c. Medium									Volume of Gas in c.c.	Percentage CO_2	Percentage H_2	Ratio CO_2/H_2
					Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite				
Type C																	
op	—	10	—	19′yoyb	2.80	4.10	3.55	—0.50	0.00	3.10	1.90	—1.10	4.00	27.95	70.4	29.3	2.40
pj	—	7	—	19yoyb	3.95	3.90	2.55	0.00	—0.90	3.40	1.35	0.00	2.95	27.48	74.2	25.5	2.95
pn	—	7	—	19′yoy	3.50	3.35	2.55	0.00	—0.85	1.55	1.10	0.00	2.55	31.34	71.4	27.3	2.61
pq	—	Slight	—	19′yoyb	4.45	4.50	3.10	0.00	—1.10	3.60	1.20	0.00	2.60	28.67	73.1	26.9	2.72
pr	—	Slight	—	19yoy	4.55	4.60	4.45	0.00	0.00	3.50	1.00	0.00	2.25	28.18	73.4	26.5	2.77
rd	—	Slight	—	19′yoy	3.35	4.25	3.60	0.00	0.00	1.80	1.20	0.00	4.05	27.75	72.5	27.4	2.65
rg	—	7	—	19yoy	3.75	4.05	3.70	0.00	0.00	2.60	0.80	0.00	1.75	28.22	71.6	28.3	2.54
rh	—	9	—	19′yoyb	3.00	3.55	3.10	0.00	0.00	3.00	0.80	—0.60	1.70	27.86	72.0	27.7	2.60
rj	—	5	—	19yoy	3.60	4.20	2.80	0.00	—0.70	3.25	2.00	0.00	2.55	28.02	70.8	29.1	2.43
rk	—	Slight	—	19′yoy	4.00	3.25	1.60	0.00	0.00	1.90	0.80	0.00	3.20	29.10	71.5	28.4	2.52
rl	—	8	—	19′yoyb	4.10	3.65	3.65	—0.70	0.00	2.30	0.70	0.00	2.90	29.57	69.8	30.1	2.32
rm	—	Slight	—	19yoyb	3.75	3.20	2.65	—0.15	0.00	2.30	1.00	0.00	1.80	29.13	71.4	28.3	2.53
rn	—	8	—	19yoy	4.35	3.85	2.70	0.00	0.00	1.45	1.25	0.00	3.00	27.62	72.3	27.5	2.63
ro	—	6	—	19yoyb	4.60	3.60	3.15	0.00	0.00	3.80	1.00	—0.95	3.20	27.33	71.3	28.7	2.48
rw	—	9	—	19yoy	3.60	3.70	3.20	0.00	—0.90	3.40	1.15	—0.80	2.05	27.23	73.1	26.8	2.73
ry	—	9	—	19′yoy	3.40	3.40	4.20	0.00	0.00	1.55	0.80	—0.70	3.05	27.48	72.0	27.7	2.60
rz	—	5	—	19′yoy	4.75	2.60	2.00	—0.10	0.00	3.65	1.30	0.00	2.50	28.73	73.0	26.9	2.73
sa	—	Slight	—	19′yoy	4.05	3.00	5.20	—0.30	0.00	0.80	1.10	0.00	1.60	27.08	73.5	26.5	2.78
sb	—	4	—	19yoyb	3.70	3.45	3.15	0.00	—1.10	2.30	1.40	0.00	1.75	27.49	72.9	26.8	2.72
sc	—	Slight	—	19′yoyb	3.35	2.90	2.30	—0.35	0.00	2.80	1.50	—1.00	3.00	27.78	72.0	27.8	2.59
sd	—	6	—	19′yoyb	4.55	3.85	3.30	0.00	0.00	3.15	1.15	—0.80	1.45	28.43	72.6	27.2	2.68
sl	—	Slight	—	19′yoyb	4.15	4.40	3.70	—0.35	0.00	3.05	1.45	—1.00	2.00	28.00	73.8	26.1	2.82
sm	—	5	—	19yoyb	3.90	4.45	3.15	—0.40	0.00	2.70	1.20	0.00	2.05	28.22	72.2	27.6	2.61
sz	—	8	—	19yoy	4.45	4.40	3.40	0.00	0.00	2.60	1.55	—1.05	3.40	28.89	72.9	26.9	2.71
tp	—	5	—	19yoy	4.40	2.75	3.60	—0.45	0.00	2.15	1.30	0.00	2.40	27.81	73.0	26.6	2.74
tq	—	9	—	19yoy	4.40	2.35	3.95	—0.25	0.00	2.60	1.30	—0.70	2.75	27.58	72.7	26.9	2.70
tr	—	9	—	19yoy	4.35	2.45	3.65	0.00	0.00	3.40	1.10	0.00	3.70	27.53	71.8	27.8	2.58
tt	—	Slight	—	19yoy	4.55	2.95	2.90	0.00	0.00	2.55	0.80	—0.85	2.30	27.83	73.4	26.3	2.79
tu	—	8	—	19yoy	4.63	2.95	3.50	0.00	0.00	2.95	1.25	0.00	3.40	27.87	73.5	26.1	2.82
ty	—	5	—	19′yoy	3.80	4.60	2.40	—0.25	0.00	3.60	1.00	0.00	2.45	28.64	69.6	29.7	2.35
tz	—	Slight	—	19yoy	4.05	4.55	3.25	—0.05	0.00	3.40	1.35	0.00	1.75	28.59	71.2	28.7	2.48
ua	—	Slight	—	19yoy	4.00	4.40	3.35	0.00	0.00	3.15	1.50	0.00	2.25	26.28	74.1	25.5	2.90
ua	....	...	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	27.81	72.8	27.0	2.70
ub	—	5	—	19yoy	4.15	4.30	3.30	0.00	0.00	2.55	1.35	0.00	2.80	28.20	72.0	27.8	2.59
uc	—	Slight	—	19yoy	4.25	3.80	3.65	—0.30	0.00	1.80	1.30	0.00	1.90	27.65	72.7	27.0	2.69
ug	—	Slight	—	19yoy	3.35	3.35	2.95	0.00	0.00	2.55	1.20	0.00	1.55	28.14	72.0	27.7	2.60
ui	—	Slight	—	19yoy	3.30	3.70	3.10	—0.30	0.00	3.15	0.95	0.00	3.80	27.98	71.5	28.3	2.52
uj	—	...	—	19yoy	3.85	3.95	3.10	—0.50	0.00	1.55	1.05	—0.75	3.30	27.75	72.9	27.0	2.70
uk	—	Slight	—	19yoyb	4.10	3.80	3.70	0.00	0.00	2.85	1.20	0.00	2.65	26.40	73.1	26.6	2.75
ul	—	Slight	—	19yoy	3.40	3.60	3.55	0.00	—0.85	3.30	0.90	—1.00	3.20	26.48	71.9	27.7	2.60
vh	—	Slight	—	19yoy	4.20	3.75	2.80	0.00	—1.05	2.20	1.20	—0.90	3.20	26.58	73.0	26.9	2.72
Type D																	
oo	—	0	—	19′yoyd	1.55	4.65	3.40	0.00	0.00	—0.70	0.85	—1.00	—1.00	30.03	70.5	29.4	2.39
ow	—	0	—	19′yoyf	1.80	5.35	2.90	0.00	—1.00	—0.10	1.15	—0.85	—1.55	31.58	71.1	28.9	2.46
ox	—	0	—	19′yoyf	1.40	5.45	3.45	0.00	0.00	—0.40	1.10	—1.00	—1.05	30.35	70.5	29.3	2.41
oy	—	0	—	19′yoyf	1.60	5.45	1.30	0.00	0.00	—0.05	1.45	—0.80	—1.70	30.68	70.1	29.7	2.36
oz	—	0	—	19′yoyf	2.20	5.25	3.55	0.00	0.00	0.00	1.10	—1.50	—1.10	30.82	70.7	29.1	2.42
oz	....	...	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	31.12	70.2	29.6	2.38
pa	—	0	—	19′yoyf	1.85	6.95	3.50	0.00	0.00	0.80	0.85	0.00	—1.50	31.79	68.7	31.1	2.21
pb	+	0	—	19′yoyf	3.85	5.70	3.65	0.00	0.00	0.50	0.60	—0.85	—1.25	30.98	69.1	31.0	2.23
pc	—	0	—	19′yoyf	1.80	5.20	1.75	—0.15	0.00	0.05	0.60	—1.10	—1.60	32.07	68.3	31.7	2.15
pi	+	0	—	19′yoyf	1.10	3.80	2.60	0.00	0.00	0.05	1.00	—1.00	—1.50	30.43	70.3	29.5	2.39

pk	—	0	—	19′yoyf	2.00	2.95	2.10	0.00	0.00	—0.40	0.95	0.00	—1.20	30.64	67.6	32.2	2.10
ps	—	0	—	19′yoyf	1.20	5.20	3.05	0.00	0.00	—0.55	0.90	—1.05	—1.45	30.85	71.4	28.4	2.52
pw	—	0	—	19′yoyf	0.95	1.95	2.55	0.00	0.00	—0.30	1.05	0.00	—1.40	30.09	70.4	29.5	2.39
px	—	0	—	19′yoyf	1.10	1.70	3.60	—0.15	—0.65	—0.60	1.05	0.00	—1.45	30.99	70.1	29.7	2.36
pz	—	0	—	19′yoyf	1.75	2.70	2.60	0.00	0.00	0.55	0.85	—1.50	—1.40	30.33	70.6	29.3	2.41
qa	—	0	—	19′yoyf	2.05	2.50	1.80	0.00	—1.15	—0.30	1.30	0.00	—1.25	30.61	71.4	28.5	2.49
qb	—	0	—	19′yoyf	1.85	5.25	4.00	0.00	0.00	0.40	1.35	—1.00	—1.50	31.33	70.7	29.1	2.42
qc	—	0	—	19′yoyf	1.85	5.15	4.15	—0.30	0.00	—0.10	1.30	0.00	—1.60	30.17	71.9	28.0	2.57
qd	—	0	—	19′yoyf	2.25	5.10	3.15	0.00	0.00	0.30	1.60	0.00	—1.55	28.84	72.2	27.8	2.60
qe	—	0	—	19′yoyf	2.10	5.20	3.15	0.10	0.00	—0.55	1.35	0.00	—1.45	28.80	70.6	29.2	2.42
qf	—	0	—	19′yoyf	2.05	5.20	2.55	0.00	0.00	0.60	1.15	0.00	—1.50	30.58	71.5	28.2	2.53
qg	—	0	—	19′yoyf	2.00	5.20	2.75	—0.35	0.00	—0.40	1.40	0.00	—1.25	30.38	72.1	27.8	2.59
qh	—	0	—	19′yoyf	1.60	2.50	1.25	0.00	0.00	0.05	1.15	0.00	—1.25	28.65	73.2	26.7	2.74
qi	—	0	—	19′yoyf	2.20	2.25	2.85	—0.20	0.00	0.55	1.20	0.00	—1.55	31.66	69.0	30.9	2.23
qj	—	0	—	19′yoyf	1.55	4.30	2.25	0.00	0.00	0.30	1.30	0.00	—1.55	32.12	70.0	29.8	2.35
qk	—	0	—	19′yoyf	1.70	3.75	1.55	0.00	0.00	—0.45	0.95	0.00	—1.40	30.59	70.3	29.6	2.38
ql	—	0	—	19′yoyf	1.50	3.50	1.35	—0.45	—1.15	—0.15	0.75	0.00	—1.65	31.80	70.3	29.6	2.38
qz	+	0	—	19′yoyf	1.70	4.40	3.95	—0.25	0.00	—0.30	1.25	—0.95	—1.50	29.33	71.9	28.2	2.55
ra	—	0	—	19′yoyf	1.50	5.20	2.95	0.00	0.00	—0.45	1.15	0.00	—1.45	29.81	69.7	30.1	2.31
rv	—	0	—	19′yoyf	1.75	4.75	3.15	0.00	0.00	—0.35	0.80	0.00	—1.40	29.74	71.0	29.2	2.43
rx	—	0	—	19′yoyf	1.65	4.90	2.50	0.00	0.00	—0.10	1.10	—1.00	—1.25	29.70	71.3	28.6	2.50
sf	+	0	—	19′yoyf	1.40	4.30	2.35	—0.40	0.00	0.00	1.20	0.00	—1.35	30.24	71.5	28.3	2.53
su	—	0	—	19′yoyf	1.90	5.35	2.95	—0.10	0.00	0.35	1.25	0.00	—1.55	29.45	71.9	27.8	2.59
tb	—	0	—	21″oyyf	1.15	2.70	3.60	0.00	0.00	—0.05	0.70	0.00	—1.35	39.94	69.7	30.1	2.32
tc	—	0	—	19′yoyf	1.30	1.00	3.50	0.00	0.00	—0.80	0.90	—0.30	—1.35	30.94	70.2	29.5	2.37
td	—	0	—	19′yoyf	1.40	0.90	3.65	0.00	0.00	—0.30	0.85	.....	—1.50	29.49	69.6	30.1	2.31
tm	—	0	—	19′yoyf	1.60	0.85	3.00	0.00	0.00	—0.20	1.90	0.00	—1.50	30.22	69.7	29.9	2.33
tn	—	0	—	19′yoyf	2.05	2.70	2.00	0.00	0.00	—0.20	0.95	0.00	—1.35	29.49	70.0	29.9	2.35
to	+	0	—	....	1.35	3.25	2.20	0.00	0.00	—0.45	1.05	0.00	—1.35	29.69	70.2	29.8	2.36
un	+	0	—	19′yoyf	2.00	5.40	4.30	—0.25	0.00	0.65	0.70	—0.60	—1.25	29.84	68.9	30.7	2.24
up	+	0	—	17′oyf	2.25	4.70	4.55	0.00	0.00	0.20	0.75	—1.05	—1.55	30.24	71.0	28.8	2.47
us	—	0	—	19′yoyf	1.50	2.30	3.40	—0.40	0.00	—0.35	0.85	0.00	—1.30	29.83	71.2	28.5	2.50
ut	—	0	—	19′yoyf	1.05	2.75	2.00	—0.40	0.00	—0.65	0.80	—0.55	—1.30	30.08	70.6	29.2	2.42
uw	—	0	—	19′yoyf	2.50	4.65	2.80	0.00	0.00	—0.75	1.15	0.00	—1.50	29.49	71.7	28.2	2.54
va	—	0	—	19′yoyf	2.40	5.20	3.60	0.00	0.00	0.35	0.95	0.00	—1.15	29.64	69.4	30.1	2.31
vb	—	0	—	19′yoyf	2.00	4.80	3.60	—0.25	0.00	—0.05	1.10	0.00	—1.45	29.97	70.0	29.8	2.35
vc	—	0	—	19′yoyf	2.35	4.55	3.20	—0.20	0.00	0.05	1.35	0.00	—0.90	29.93	69.1	30.5	2.26
vg	—	0	—	19′yoyf	2.10	4.70	3.30	0.00	0.00	0.05	0.90	0.00	—0.45	30.63	69.9	30.0	2.33
ud	+	0	—	19″yoyf	2.10	1.40	4.20	0.00	0.00	1.90	0.90	0.00	—1.60	31.19	68.7	31.0	2.22
vi	—	0	—	19′yoyf	2.50	3.40	4.00	0.00	0.00	1.00	1.15	0.00	—1.00	30.46	70.5	29.3	2.41
re	+	0	—	*	2.90	3.55	0.85	0.05	—0.75	2.60	0.35	—0.50	—1.30	26.47	68.5	31.2	2.20
ri	+	0	—	*	3.50	3.40	1.05	0.45	—0.45	3.70	0.70	—0.40	—1.05	21.26	67.0	32.6	2.05
ri	....	...	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	17.48	56.3	43.6	1.29
ri	....	...	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	20.72	66.1	33.7	1.96
on	—	0	—	19′yoyf	1.45	5.10	1.75	0.00	—0.95	0.15	0.85	—0.95	2.35	31.09	69.5	30.4	2.28
pe	—	0	—	21′oyyd	1.15	3.95	2.40	0.00	0.00	—0.35	1.05	0.00	2.35	30.04	70.7	29.4	2.40
qm	—	0	—	19′yoyf	1.20	3.05	0.85	0.00	0.00	—0.10	1.25	0.00	0.80	32.25	70.4	29.4	2.40
qn	—	0	—	19′yoyf	1.80	5.10	1.20	—0.30	0.00	—0.35	0.95	0.00	1.40	31.84	69.0	30.9	2.23
qo	—	0	—	19′yoyf	2.55	4.20	3.30	0.00	0.00	—0.20	0.90	0.00	1.35	31.33	68.8	31.1	2.21
qp	—	0	—	19′yoyf	1.70	5.00	3.05	0.00	0.00	—0.45	0.65	0.00	1.25	29.62	68.5	31.5	2.18
qq	—	0	—	19′yoyf	1.70	5.20	4.30	0.00	0.00	—0.60	0.75	0.00	0.65	30.49	69.4	30.0	2.31
qr	—	0	—	19′yoyf	2.20	4.80	2.05	0.05	0.00	—0.15	1.15	0.00	2.75	31.10	69.9	29.8	2.34
qu	—	0	—	19′yoyf	1.70	4.40	0.95	—0.10	0.00	0.20	1.15	0.00	2.95	31.08	68.8	31.1	2.22
um	+	0	—	17′oyf	2.25	4.85	4.40	0.00	0.00	0.00	0.80	—0.50	1.55	29.56	70.5	29.3	2.40
ur	—	0	—	19′yoyf	2.05	4.50	2.85	0.00	0.00	—0.10	0.80	0.00	1.80	29.84	71.1	28.6	2.49
uu	—	0	—	19′yoyf	3.25	4.85	2.75	—0.10	0.00	0.10	0.95	0.00	1.35	30.63	69.4	30.5	2.27
uv	+	0	—	19′yoyf	2.15	5.35	3.40	—0.35	0.00	0.05	1.05	0.00	1.50	29.71	71.1	28.5	2.49
ux	—	0	—	19′yoyf	2.65	5.95	3.00	0.00	0.00	0.25	1.35	0.00	1.45	30.29	70.3	29.5	2.38
uz	—	0	—	19′yoyf	2.70	5.05	3.25	—0.30	0.00	—0.25	1.20	0.00	1.50	29.24	70.2	29.7	2.37

* Growth could not be removed from agar.

TABLE 3.—*Continued*

Culture	Capsule	Liquefaction of Gelatin in mm.	Indol from Tryptophan	Pigment	Acid Fermentation Reactions Expressed in Percentages									Volume of Gas in c.c.	Percentage CO_2	Percentage H_2	Ratio $\frac{CO_2}{H_2}$
					Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite				
os	—	0	—	21″oyyf	1.95	2.25	2.35	—0.25	0.00	1.50	1.20	4.70	—1.45	31.08	68.7	31.2	2.20
ot	—	0	—	19′yoyf	2.50	3.35	3.10	—0.35	0.00	1.05	0.80	4.65	—1.50	31.55	68.1	31.9	2.14
qv	—	0	—	21″oyyf	1.30	5.00	2.00	0.00	0.00	0.75	1.05	4.55	—1.50	32.02	68.3	31.3	2.18
qw	—	0	—	19′yoyf	1.90	5.00	1.45	0.00	0.00	0.15	0.95	4.40	—1.70	30.33	69.4	30.5	2.27
qw	....	...	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	30.27	68.9	31.0	2.23
qx	+	0	—	19′yoyf	1.65	5.05	3.35	0.00	0.00	—0.50	0.90	5.45	—1.60	31.25	68.5	31.4	2.19
om	+	0	—	None	2.75	4.15	1.95	5.85	—0.70	0.85	0.90	—0.60	—1.10	28.81	69.5	30.4	2.28
ov	+	0	—	None	3.25	4.20	1.55	5.20	—0.70	0.60	2.65	—0.70	—1.10	29.50	70.2	29.2	2.40
pv	+	0	—	19′yoyf	2.20	4.50	2.10	5.20	—0.75	1.20	1.20	—0.60	—1.10	28.29	71.8	28.1	2.55
py	+	0	—	None	2.40	4.90	3.50	5.50	—0.75	1.70	3.20	—0.80	—0.70	28.77	70.1	29.7	2.36
rq	+	0	—	**None**	2.40	5.05	3.55	6.20	—0.75	0.35	3.45	—0.70	—1.05	29.69	70.1	29.7	2.36
sx	+	0	—	17″oyd	3.45	5.10	2.45	4.35	—0.45	1.55	2.25	—0.70	—0.60	30.23	68.6	31.2	2.20
pd	+	0	—	None	4.15	4.35	4.00	5.65	—0.90	0.80	0.95	—0.60	0.95	30.01	69.6	30.3	2.30
rb	+	0	—	None	2.65	4.10	2.50	5.55	—0.70	0.35	2.35	—0.55	0.70	28.47	67.2	32.8	2.04
rc	+	0	—	None	2.90	4.30	3.40	2.60	—0.75	0.45	3.30	—0.60	0.85	29.14	68.0	31.8	2.14
tx	+	0	—	21″oyyf	2.55	3.00	1.80	7.00	—0.85	—0.05	3.95	4.25	—0.90	29.63	68.0	31.8	2.14
pf	+	0	—	None	2.80	3.50	3.80	4.85	4.75	0.50	1.40	—0.50	0.35	30.54	69.5	30.0	2.32
pg	+	0	—	None	2.95	3.95	2.80	5.65	4.50	0.55	0.75	—0.60	0.65	29.69	70.8	29.0	2.44
ph	+	0	—	None	2.75	4.35	4.00	5.40	4.30	0.95	0.70	—0.55	0.65	29.71	70.5	29.5	2.39
rr	+	0	—	None	3.30	5.10	2.40	4.10	4.55	1.25	1.15	—0.50	—1.10	29.74	69.1	30.8	2.24
rs	+	0	—	None	2.40	5.00	3.30	3.15	4.30	0.65	3.40	—0.80	—1.10	29.52	71.0	29.1	2.44
ru	+	0	—	None	3.20	4.00	3.70	3.35	3.30	1.00	2.00	—0.60	—1.20	28.87	70.2	29.6	2.37
sy	+	0	—	21″oyyf	3.40	5.25	1.90	2.45	4.70	0.15	3.25	—0.70	—1.15	30.75	67.0	32.4	2.08
ta	+	0	—	21″oyyf	3.35	5.00	2.90	2.95	4.85	1.25	1.15	—0.30	—1.15	29.77	69.0	30.9	2.24
tl	+	0	—	21″oyyf	2.45	4.85	2.00	5.45	4.55	0.55	.....	—0.70	—1.10	29.59	68.8	30.9	2.23
Type E																	
pt	+	0	+	21″oyyf	2.82	5.10	3.75	5.35	—0.90	1.65	3.20	4.40	1.10	29.58	69.1	30.7	2.25
pu	+	0	+	21″oyyf	2.45	5.40	4.40	5.55	—0.90	2.45	3.30	4.95	0.65	29.00	67.0	33.2	2.02
pu	....	...	....	....	.....	.....	.....	.....	.....	.....	.....	.....	.....	31.03	67.4	32.2	2.09
qs	+	0	+	19′yoyf	3.30	5.05	2.83	4.60	—0.95	2.35	1.75	4.50	1.15	28.01	67.9	31.8	2.14
qt	+	0	+	None	3.30	4.90	1.85	1.50	—0.95	3.25	3.45	4.70	2.30	26.73	71.0	28.9	2.46
rp	+	0	+	None	3.15	5.90	3.60	1.25	—0.60	1.00	3.15	4.50	0.95	30.29	67.8	31.8	2.13
po	+	0	+	19′yoyf	2.60	3.65	3.15	5.45	4.80	1.55	2.80	4.45	2.35	30.80	69.0	30.9	2.23
rt	+	0	+	19′yoyf	3.00	6.45	2.65	2.10	2.40	3.70	3.50	5.55	1.15	29.21	69.9	29.9	2.34
qy	+	0	+	None	2.80	5.35	2.70	4.70	4.80	0.80	3.35	5.25	—1.45	30.16	68.2	31.5	2.17
Type F																	
se	+	0	—	19′yoyf	—0.10	0.80	0.00	0.00	0.00	1.30	0.80	—0.90	—1.25	31.95	67.3	32.4	2.08
sg	+	0	—	19′yoyf	—0.70	0.40	0.00	—0.40	0.00	0.25	0.85	0.00	—0.35	31.15	66.0	33.8	1.95
sh	+	0	—	21″oyyf	—0.85	0.55	0.00	—0.45	0.00	0.35	0.85	0.00	—1.50	29.31	70.0	29.7	2.36
si	+	0	—	21″oyyf	—0.40	0.50	0.00	—0.45	—1.05	0.95	0.85	—0.85	—1.00	31.40	66.0	33.3	1.98
sn	+	0	—	19′yoyf	—0.70	0.20	0.00	0.00	0.00	1.50	0.85	—0.95	—1.15	31.95	67.8	32.0	2.12
so	+	0	—	....	—0.70	0.20	0.00	—0.30	0.00	0.55	1.25	0.00	—1.50	31.82	67.9	31.9	2.12
sp	+	0	—	19″yoyf	—0.90	0.30	0.00	—0.15	0.00	0.05	0.90	—1.00	—1.50	32.31	66.1	33.6	1.97
sq	+	0	—	19′yoyf	—0.25	1.00	0.00	0.00	—1.00	0.35	0.95	0.00	—1.50	32.75	66.1	33.7	1.96
sr	+	0	—	19′yoyf	—0.60	0.20	0.00	—0.25	0.00	0.75	1.05	—0.60	—1.40	31.70	66.7	33.0	2.02
ss	+	0	—	19′yoyf	—0.65	0.05	0.00	0.00	—0.45	1.60	1.15	—0.90	—1.15	31.03	67.9	31.9	2.13
uf	+	0	—	21″oyyf	—0.40	0.20	0.00	—0.20	0.00	0.50	0.60	0.00	—1.50	31.54	66.6	33.1	2.01
uh	+	0	—	19′yoyf	—0.45	0.00	0.00	0.00	0.00	1.10	0.85	—0.90	—1.40	32.06	66.3	33.4	1.98
uo	+	0	—	17′oyf	—0.80	0.05	0.00	0.00	0.00	0.50	1.00	0.00	—1.15	31.15	68.1	31.3	2.18

fall the average results of the sixteen analyses of the gas produced by the eight low ratio cultures, as did those of 182 of the analyses of the gas produced by the 149 low ratio bacteria isolated from bovine feces. The average ratio in that series was $CO_2 : H_2 = 1.06$, and in this series it was again 1.06.

In like manner, the absence of hydrogen in the gas produced by seven of the cultures characterized another group, shown in Chart 1 by the dots on the base line.

In the case of organisms giving the high ratios, the distinction from the other two groups was sharp, but the homogeneity within the group was not so clear. Similar high ratios with similar, though greater, variations were found in the analyses reported in our study of the gas-producing bacteria isolated from milk and milk products.[14] We had hoped that improvements in our technic, especially the employment of a simpler medium, made with accuracy and without the usual vain attempts to correct its "reaction," would result in the appearance of greater uniformity among the high ratios, as it did among the low ratios. This hope has thus far been dispelled, and we are forced to fall back upon the suggestion published in our first paper—that we have to do with two gas-producing reactions, one of which furnishes carbon dioxid alone. If this be true, then the end point of one reaction will have to depend strictly upon the other in order to avoid variations in the ratio $CO_2 : H_2$ with small changes in conditions.

TABLE 4

AVERAGE VALUES IN GAS ANALYSES OF THREE GROUPS

Group	Total Gas, c.c.	CO_2 c.c.	H_2 c.c.	N_2 c.c.	CO_2 %	H_2 %	N_2 %	Ratio CO_2/H_2
High ratio.................	29.50	20.67	8.75	0.08	70.1	29.7	0.2	2.36
Low ratio..................	12.41	6.37	6.00	0.04	51.3	48.3	0.4	1.06
∞ ratio....................	5.50	5.50	.....	Trace	100 ?	.....	Trace	∞

A few of the colon cultures isolated from cow feces which were described in our last paper, were carried for a time as usual on infusion agar and then a subculture of each was made in milk. These subcultures in milk were carried at 20 C. for thirty-three weeks with a transfer every seventh day. At the end of this period, gas determinations were made with these subcultures in a medium of the same composition that a year before had been used with the original cultures. The

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[14] Jour. Infect. Dis., 1914, 14, p. 411.

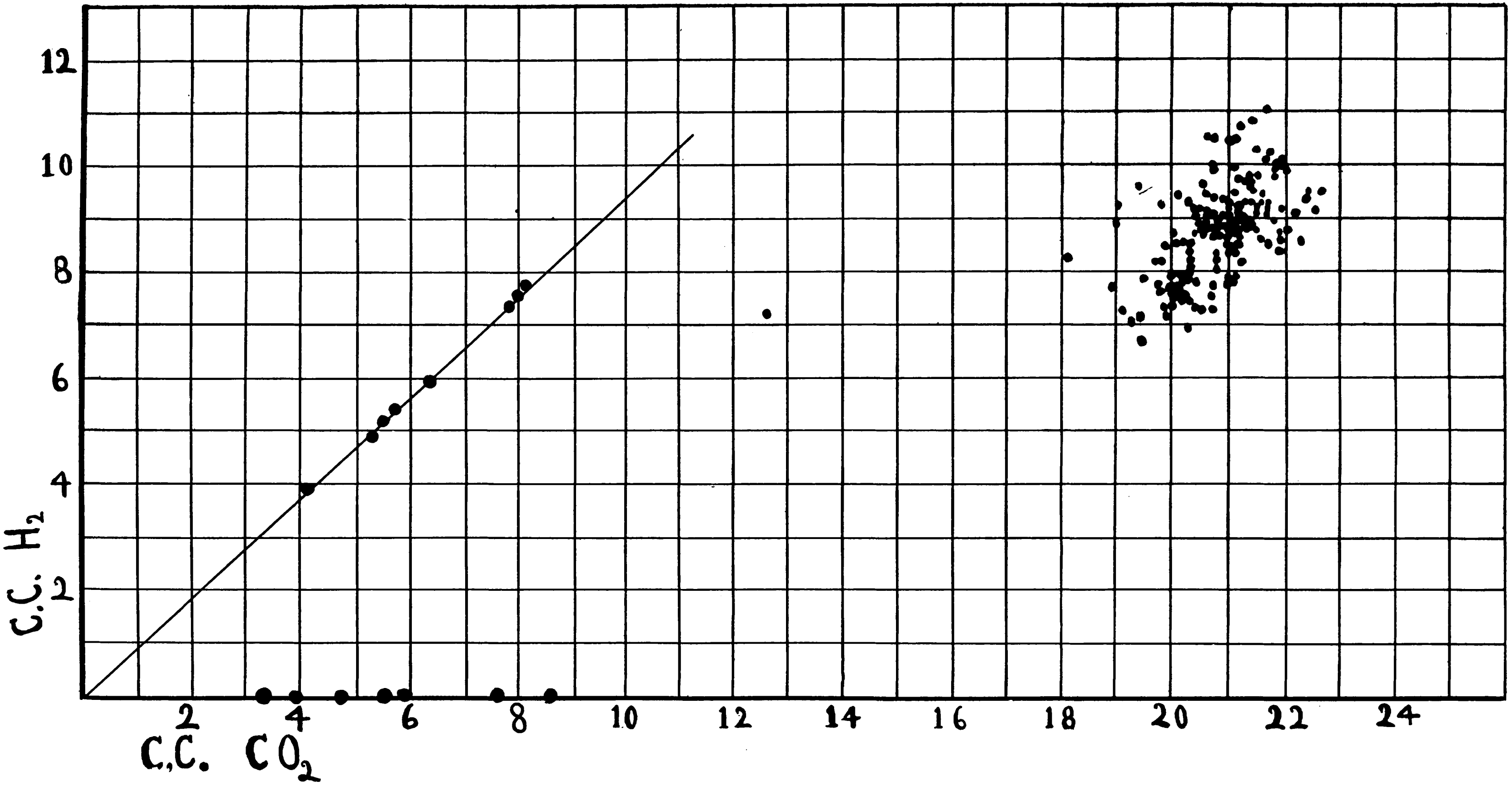

Chart 1.—Showing the differences in gas-production of the "high ratio," "low ratio," and ∞ groups of bacteria of the colon-aerogenes type.

gas determinations with the original young cultures and the old subcultures are given in Table 5. Their agreement speaks for itself.

CORRELATION OF RESULTS

The possibility of dividing this collection of cultures into three major groups on the basis of the carbon dioxid-hydrogen ratio has already been pointed out. The ability to bring about a certain fermentation with certain definite end products is evidently firmly fixed, and is more a mark of relationship than the ability to utilize any particular substance in bringing about this fermentation. In our earlier papers, it has been shown that the production of carbon dioxid and hydrogen in a certain definite proportion is closely correlated with other physiologic characteristics and is therefore a proper basis for subdivision.

TABLE 5

GAS PRODUCTION OF YOUNG AND OLD COLON CULTURES

Date	Culture	Total Gas, c.c.	CO_2 %	H_2 %	Ratio CO_2/H_2
1913	jh............................	13.99	51.1	48.2	1.06
1914	....	14.10	51.4	48.1	1.07
1913	jm............................	11.64	51.3	48.7	1.05
1914	....	13.05	52.1	47.8	1.09
1913	jn............................	15.07	50.8	48.6	1.04
1914	....	16.09	52.1	47.7	1.09
1913	ma............................	13.42	51.6	47.8	1.08
1914	....	13.80	51.8	47.7	1.09
1913	nh............................	16.22	53.0	46.8	1.13
1914	....	14.41	51.9	48.1	1.08

One of the divisions of this collection, made on this basis, is separated radically from the other two groups by its failure to produce hydrogen. The general characteristics of this group have been brought together in Table 2, and summarized briefly in Table 8 (Type B).

This table shows that the cultures included in this group have many characteristics in common, in addition to the gas production. A capsule was observed on one culture only; all liquefied gelatin; only one produced indol from tryptophan; all fermented saccharose; only one fermented lactose or raffinose; glycerin was fermented by five of the seven and adonite by four, while none were able to utilize starch, inulin, or dulcite. Nearly all the cultures produced decided pigment, but there was some variation in the color. The peculiar gas production, the failure to ferment lactose, together with the liquefaction of gelatin, which in many cases is active, may be taken as sufficiently distinctive to separate these cultures from the colon group. However, any conclusions based on a study of seven cultures only must necessarily be tentative.

In our study of the colon cultures from bovine feces, it was found that they were almost exclusively of a type adhering very closely to the carbon dioxid-hydrogen ratio of 1.06. Eight of our grain cultures agreed with this type in that the gas ratio was identical. The characteristics of this group, which for convenience we will designate as Type A, are tabulated in Table 1 and summarized in Table 8.

TABLE 6

Positive Reactions Arranged by Types

Type	Capsule	Gelatin	Indol	Saccharose	Lactose	Raffinose	Starch	Inulin	Mannite	Glycerin	Adonite	Dulcite
A	1* 12.5	0 0	4 50.0	2 25.0	8 100.0	6 75.0	0 0	1 12.5	8 100.0	2 25.0	3 37.5	4 50.0
B	1 14.3	7 100.0	1 14.3	7 100.0	1 14.3	1 14.3	0 0	0 0	7 100.0	5 71.4	4 57.1	0 0
C	0 0	40 100.0	0 0	40.0 100.0	40 100.0	40 100.0	0 0	0 0	39 97.5	32 80.0	0 0	40 100.0
D	32 30.5	0 0	0 0	90 100.0	90 100.0	87 96.4	19 21.1	9 10.0	11 12.2	51 56.6	6 6.7	13 14.4
E	8 100.0	0 0	8 100.0	8 100 0	8 100.0	8 100.0	8 100.0	3 37.5	7 87.5	8 100.0	8 100.0	5 62.5
F	13 100.0	0 0	0 0	0 0	13 100.0	0 0	0 0	0 0	5 38.4	4 30.8	0 0	0 0

* The first number = number of cultures; the second, percentage of total.

In a general way, the fermentation tests agreed also; but half of these cultures failed to produce indol, while the fecal organisms gave a positive reaction without exception. The difference in the pigment formation was even more marked, as is shown in Table 7. Six of the eight fall on the pure color of the 19″ yoy plate, while one is classed as one tint lighter, and one as one shade darker. The decided yellow color of these cultures was readily noticeable, even in the comparatively young culture. This is in marked contrast to the colorless cultures of fecal origin.

These two groups, Types A and B, altho they were sharply defined, were numerically insignificant, making together only 15 of the 166 cultures. The remaining 151 cultures may be thrown together into a group with gas ratios varying from 1.91 to 3.00. While it is evident that this is a heterogeneous group, there is no evidence of lines of separation on the basis of gas ratios as they are arranged in Chart 1.

We may make a tentative division, however, by grouping together the forty feeble liquefiers. Reference to Table 6 shows that these cultures, which we have designated Type C, all fermented saccharose, lactose, raffinose, and dulcite, and, with one exception, all fermented mannite. They failed completely to form a capsule, produce indol, or ferment starch, inulin, or adonite. There was a very close agreement among themselves and in some respects a sharp differentiation from the other types. This is perhaps shown more clearly in Chart 2, which is a graphic representation of Table 6.

The type represented under D was the most numerous of the collection, including ninety cultures. It was distinguished from Type C, not only by the gelatin reaction, but also by the failure to ferment mannite and dulcite and, to a lesser extent, by the action on glycerin. A capsule was observed on 30 percent of the D cultures against none in the C type.

There remain two small groups, E and F, represented by eight and thirteen cultures, respectively. The activity of the E type is well represented in Chart 2, which shows nearly all the reactions, with the exception of gelatin, on the positive side of the line. A considerable proportion of the cultures fermented even the more resistant inulin. On the other hand, the F type failed almost completely on all of the tests with the exception of lactose. It should be observed however that some of these reactions might have been obscured by the secondary alkaline fermentation. A capsule was observed in all the cultures included in these two groups.

On the basis of the tentative grouping shown in Table 6 and Chart 2, frequency polygons have been constructed showing the distribution of cultures included in the high ratio types, C, D, E, and F, on the basis of the carbon dioxid-hydrogen ratio. This is shown in Chart 3. Altho the types overlap one another to some extent, each is confined to a definite part of the figure. The extreme low side is occupied by Type F, while, partly overlapping it, but distinctly higher, is Type E. On the extreme high side is Type C, while the center is occupied almost exclusively by Type D. This additional evidence of relationship strengthens very materially the tentative grouping made on the basis of the fermentation reactions more commonly employed.

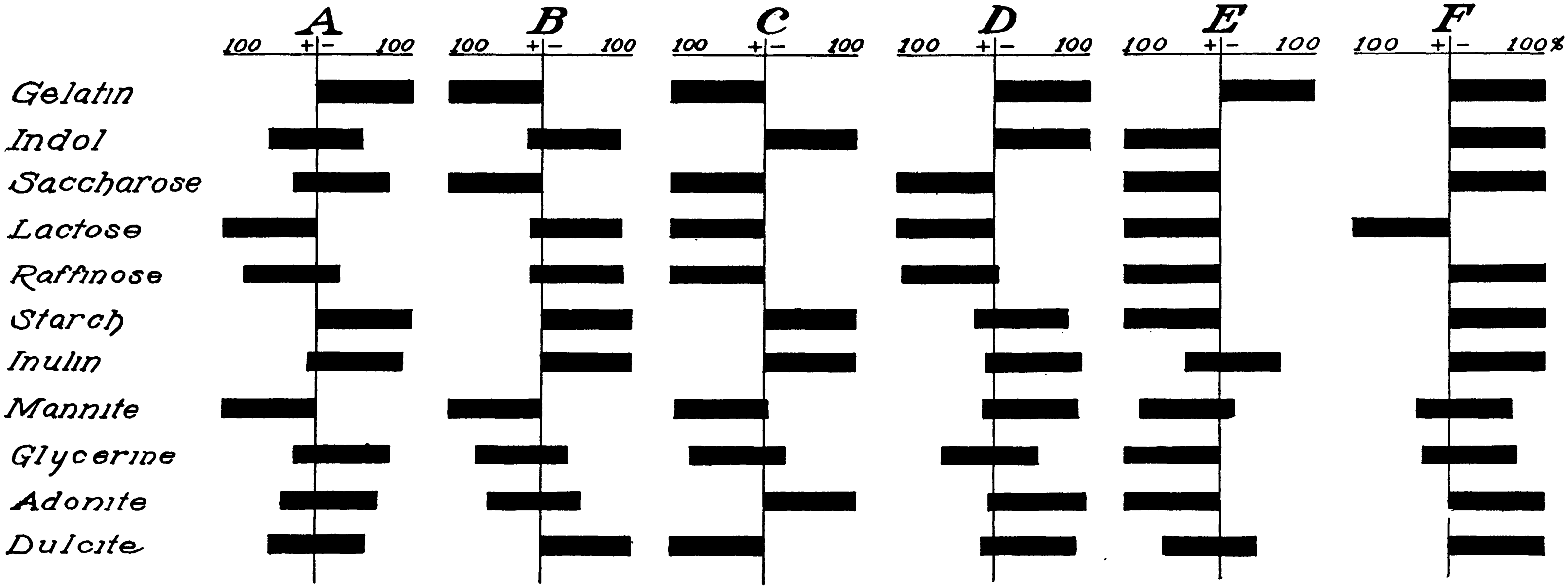

Chart 2.—Showing the differences in fermentation reaction of the A, B, C, D, E, and F types of the colon-aerogenes group. A positive reaction is plotted to the left, a negative one to the right of the median lines.

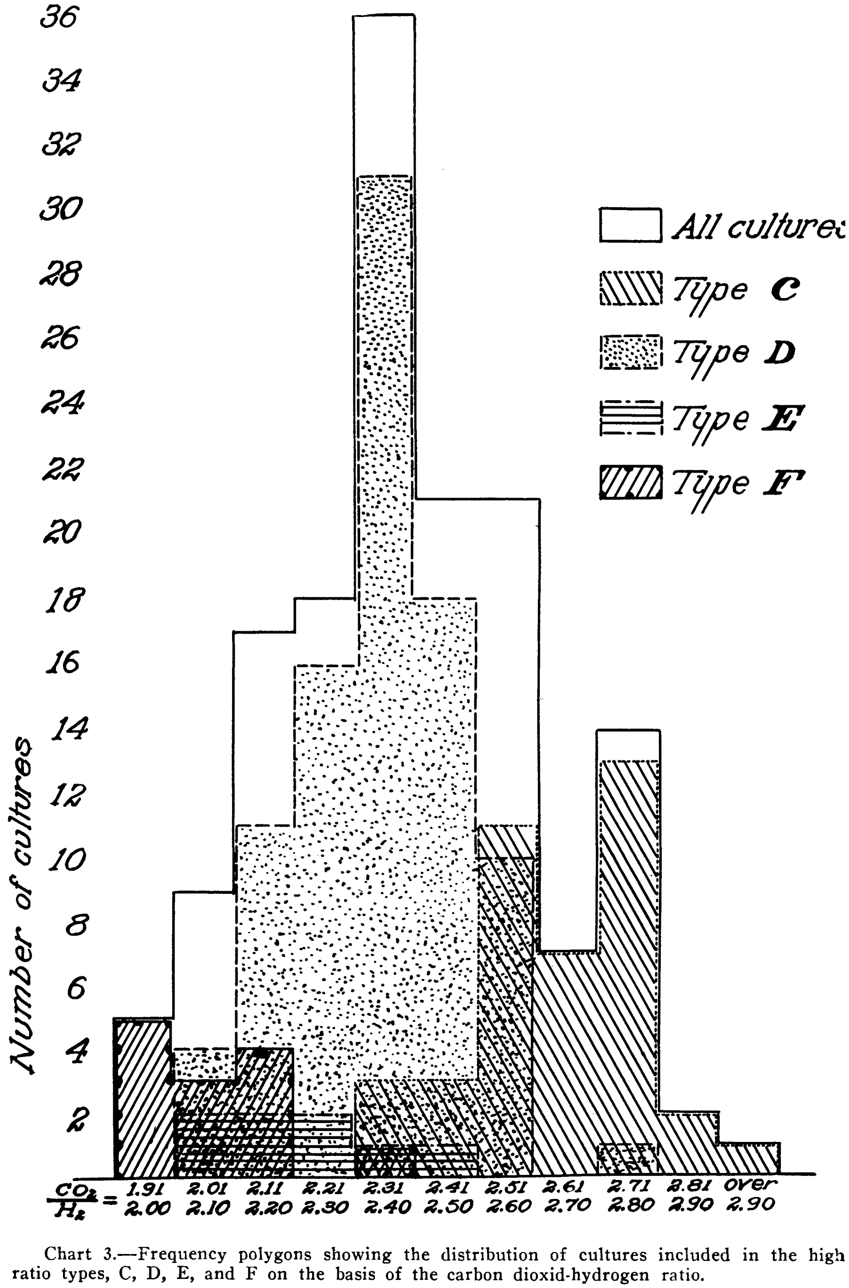

Chart 3.—Frequency polygons showing the distribution of cultures included in the high ratio types, C, D, E, and F on the basis of the carbon dioxid-hydrogen ratio.

In the previous work on the colon-aerogenes group, the formation of pigment was not considered a factor in the classification. This may have been because the types usually studied are nearly, or quite, free from pigment. Even a casual examination of the grain series showed that a large proportion of them were pigment formers to some degree. Usually this was so slight that the culture would be described as white; but when it was grown for some time at a low temperature and the growth spread on white paper, it was very easy to match these with standard colors. The results of these comparisons are tabulated in Table 7.

The degree of correlation in the pigment formation was greater than would appear at first glance because of the difficulty in arranging the table to bring together those colors most likely to be mistaken for one another. The groups A, B, and C were distinctly pigmented, so much so that it would have been noticeable in the ordinary agar culture. Types D, E, and F, on the other hand, had only a small amount of pigment and would usually have been considered white. The A type, which is the one with the colon ratio, had six of the eight cultures under the pure color of 19″ yoy, while one culture varied from it by one shade, and one by one tint. The seven cultures of Type B were somewhat scattered, but of the forty cultures of Type C, twenty agreed with the pure color of 19 yoy, six differed from this by one shade, and fourteen were matched with colors which differed from these two by the addition of a small amount of neutral gray. The D cultures had only a small amount of pigment, practically all falling in the f-tints, which are those nearest the white. It is not easy for the ordinary eye to distinguish between the light-buff, cream-color, car-

TABLE 7

Distribution of Cultures by Pigment Formation

Numbers and letters in heads refer to plates in Ridgeway's Color Standards and Nomenclature.

		17oy	17′oy	17″oy	19yoy		19′yoy				19″yoy				21″oyy	
	No Pigment	Cadmium Yellow	f Light Buff	d Pinkish Buff	b Apricot Yellow	Light Cadmium	f Cream Color	d Naples Yellow	b Mustard Yellow	Primulin Yellow	f Cartridge Buff	b Chamois	Honey Yellow	i Isabella Color	f Ivory Yellow	d Colonial Buff
Type A....	..	..	..	..	..	..	..	..	..	..	..	1	6	1	..	..
Type B....	..	2	1	2	..	..	1	..	..	1	..	..	..	..	..	..
Type C....	..	..	..	..	6	20	..	..	7	7	..	..	..	..	..	..
Type D....	13	..	2	..	..	..	62	1	..	..	1	..	..	..	7	1
Type E....	3	..	..	..	..	..	3	..	..	..	..	..	..	..	2	..
Type F....	..	..	1	..	..	..	7	..	..	..	2	..	..	..	3	..

tridge-buff, and ivory-yellow with which the cultures of this type were matched. Types E and F followed D very closely.

Table 8 is arranged to show the reactions of the typical culture of each of the six types. In Types A, E, and F, the number of cultures examined was not large enough to establish a type with any finality, but the characteristics of B were so radically different that there is no question about its separation from the others. Type C, with its forty cultures agreeing with one another almost like duplicates, may be said to be well established. The large group, D, is composed of ninety cultures with a relatively small number of variants. In comparing these types with those in other collections, it should be remembered that the gas ratio may vary with conditions and that gas determinations made with Smith tubes are not comparable with those made by Keye's method.

TABLE 8
REACTIONS OF TYPE CULTURES

	A	B	C	D	E	F
Ratio $\frac{CO_2}{H_2}$	1.06	∞	2.51-2.80	2.20-2.50	2.10-2.30	1.90-2.20
Pigment....................	Honey yellow	Cadmium yellow, pinkish buff	Light cadmium	Cream color	Cream color	Cream color
Capsule....................	—	—	—	—	+	+
Indol from tryptophan....	±	—	—	—	+	—
Gelatin.....................	—	+	+	—	—	—
Saccharose................	—	+	+	+	+	—
Lactose.....................	+	—	+	+	+	+
Raffinose...................	+	—	+	+	+	—
Starch......................	—	—	—	—	+	—
Inulin.......................	—	—	—	—	±	—
Mannite.....................	+	+	+	—	+	±
Glycerin....................	±	+	+	±	+	±
Adonite.....................	±	+	—	—	+	—
Dulcite......................	±	—	+	—	+	—

CONCLUSIONS

By careful selections of the manifestations of the physiologic activities most characteristic of a particular group of bacteria, it is possible, by the collection of sufficiently accurate data, to bring together the closely related cultures into natural species, or varieties, and to establish lines of separation as sharply defined as is possible in view of the overlapping which actually exists in nature. The stability of this grouping cannot be shaken by evidence of variation in some of the characteristics used for purposes of classification. The fact that these cultures with their peculiar grouping of characteristics occur in large numbers in similar habitats in widely separated localities is almost conclusive evidence that the groups into which they fall are valid. Species so established cannot be identified or separated from one another by the turn of a single characteristic. Natural groups of bacteria are bound together by the common possession of a certain combination of characteristics, no one of which is absolutely fixed. To attempt to separate a family into species by dividing all those that respond to some arbitrary test from those that fail to respond, is certain, in many cases, to remove a culture from its natural relatives. It may seem that we have violated this principle in making primary separations on the basis of the gas ratio. It should be remembered however that this is only one of numerous correlated characteristics which separate the major group into distinct minor groups.

Within the limits of the colon-aerogenes group, there are several well-defined sub-groups, or species. One of these is peculiar to the digestive tract, or at least to the bovine digestive tract, and is characterized by the relatively low gas production, a carbon dioxid-hydrogen ratio under definite conditions of 1.06, the uniform production of indol from tryptophan, and the fermentation of dulcite.

It would not be safe to say that the bacteria discussed in this paper had their normal habitat on grains and on grains exclusively. The method of collection, with the possible exception of the green oats, did not exclude the possibility of contamination, and the fact that the grains were thoroughly dried has some bearing on the question. Against the supposition that these bacteria were mere accidental contaminations carried by the air dust and probably of fecal origin, was the very uniform and marked difference between the grain types and the bovine type. It should also be noted that of the fifteen cultures obtained from two samples of green oats, four belonged in Type B, one in Type C, and ten in Type D.

It is significant that the same definite type of bacteria should be found on grains coming from so many widely separated sources and that this type should differ in so many respects from that found in bovine feces.

Of the six types into which this collection of cultures has been divided, three may be considered well established. One of these, B, occurred only a few times; but on account of its peculiar gaseous fermentation, its failure to ferment lactose, and the liquefaction of gelatin, it may be considered as a distinct variety which has at best only a slight connection with the colon-aerogenes group. Notwithstanding this marked difference, certain lactose-fermenting strains may easily be mistaken for Bacillus coli. Indeed, one of the cultures furnished us by Mr. Kligler as an atypical colon belonged in this group.

The validity of including the group designated as Type C in the colon-aerogenes group may be questioned. In the classification of the colon group adopted by the Laboratory section of the American Public Health Association,[15] failure to liquefy gelatin in fourteen days is given as a characteristic of the bacillus coli group, altho one variety is given which liquefies gelatin in twenty days. So far as published descriptions permit us to make comparisons, this type agrees with Bacillus cloacae. Jordan,[16] who first described this organism, considered that it belonged to the proteous group, but many later writers seem inclined to place it with the colon-aerogenes group. In any case, the cultures which we have included in this group are typical of the slow liquefiers, which may be included in the colon-aerogenes group without question. The gelatin liquefaction is so slow and the pigment production so slight that these characters could easily be overlooked. It is not improbable that they may, at times, be entirely lost.

The type D, if the number of cultures in this collection may be taken as an indication, is the most common of the grain varieties. It differs from C in gelatin liquefaction, and mannite and dulcite fermentation, and from E in indol production, and starch, mannite, adonite, and dulcite fermentation. In this group we find that something over one-half the cultures adhere very closely to the type. The remaining cultures may be classed as variants from the type. It should be noted however that these variations do not occur in single characteristics, but that two or three characteristics of a culture vary together. In some cases, for instance, the presence of a distinct capsule is correlated with an entire absence of pigment, a strong fermentation of starch and inulin, and a failure to ferment dulcite. That there are usually several variants with identical characteristics may be due in some cases to the fact that they all came from the same sample, but it frequently happened that identical variants came from widely separated sources. The question of considering these as variants or as a distinct type, is largely a matter of opinion. With the information now available, we prefer to consider them as variations from the type. This type may be identified perhaps with the ordinary conception of Bacillus aerogenes. Kligler describes Bacillus aerogenes as lactose +, saccharose +, salicin +, glycerin +, gelatin —, indol ∓, a description agreeing for the most part with our Type D, except that not one of our ninety cultures gave a positive test for indol. However, the particular name which we shall apply to these groups, or their identity with previously described species, is of no great importance at this time. The really significant fact established is the existence of clearly defined varieties, or sub-groups, of this family, and their occurrence in a definite habitat.

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[15] Standard Methods for the Examination of Water and Sewage, 1912, p. 80.
[16] Jour. Hyg., 1903, 3, p. 1.

SUMMARY

The work of other investigators has shown that bacteria of the colon type occur frequently on grains, fruits, and grasses; but these are usually assumed to be identical with the fecal type, if not merely contamination with fecal matter carried as dust.

Our own investigations have demonstrated that the colon bacteria of the bovine intestine belong, apparently almost without exception, to a single, sharply defined type characterized by the production of hydrogen and carbon dioxid in the proportion of 1 to 1.06.

A collection was made of 166 cultures of colon-like bacteria obtained from thirty-three samples of dried grains coming from various states, and from two samples of green oats. On the basis of the gas produced in the anaerobic fermentation of dextrose, these cultures may be divided into three general groups:

Seven cultures produced hydrogen only. These cultures liquefied gelatin and did not produce indol. They usually fermented saccharose, mannite, glycerin, and sometimes adonite, but usually failed to ferment lactose, raffinose, starch, inulin, and dulcite. They formed a yellow pigment on agar.

Eight cultures gave a gas ratio of 1.06 in agreement with the fecal type, but differed in the failure of half the cultures to form indol, in their failure to ferment glycerin, and particularly in their production of a yellow pigment.

All of the remaining cultures gave gas ratios varying from 1.90 to 3.00.

Of these 151 cultures, forty liquefied gelatin slowly and fermented saccharose, lactose, raffinose, and dulcite. They formed a yellow pigment corresponding to the light cadmium of Ridgway's plates. Nearly all fermented mannite and glycerin. All failed to form indol and to ferment starch, inulin, and adonite. The carbon dioxyd-hydrogen ratio varied from 2.30 to 3.00.

Ninety cultures constituted one type, of which all fermented saccharose, lactose, and raffinose and about one-half fermented glycerin. All failed to liquefy gelatin, and practically all failed to ferment starch, inulin, mannite, adonite, and dulcite. The carbon dioxyd-hydrogen ratio ranged from 2.00 to 2.60.

Eight cultures were grouped together by the formation of indol and particularly by their marked fermentative ability. Practically all the cultures fermented all the test substances used. The gas ratio was between 1.90 and 2.20.

Thirteen cultures differed from the other types in failing to liquefy gelatin, form indol, or to ferment any of the test substances except dextrose and lactose.

None of the types found on grains agreed with the characteristic flora of bovine feces. Even the type with corresponding gas ratio was easily distinguished by its pigment formation.